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Procalcitonin and midregional proatrial natriuretic peptide as biomarkers of subclinical cerebrovascular damage: the northern manhattan study

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Abstract: **BACKGROUND AND PURPOSE:** Chronic infections and cardiac dysfunction are risk factors for stroke. We hypothesized that blood biomarkers of infection (procalcitonin) and cardiac dysfunction (midregional proatrial natriuretic peptide [MR-proANP]), previously associated with small vessel stroke and cardioembolic stroke are also associated with subclinical cerebrovascular damage, including silent brain infarcts and white matter hyperintensity volume. **METHODS:** The NOMAS (Northern Manhattan Study) was designed to assess risk factors for incident vascular disease in a multiethnic cohort. A subsample underwent brain magnetic resonance imaging and had blood samples available for biomarker measurement (n=1178). We used logistic regression models to estimate the odds ratios and 95% confidence intervals (95% CIs) for the association of these biomarkers with silent brain infarcts after adjusting for demographic, behavioral, and medical risk factors. We used linear regression to assess associations with log-white matter hyperintensity volume. **RESULTS:** Mean age was 70±9 years; 60% were women, 66% Hispanic, 17% black, and 15% were white. After adjusting for risk factors, subjects with procalcitonin or MR-proANP in the top quartile, compared with the lowest quartile were more likely to have silent brain infarcts (adjusted odds ratio for procalcitonin, 2.2; 95% CI, 1.3-3.7 and for MR-proANP, 3.3; 95% CI, 1.7-6.3) and increased white matter hyperintensity volume (adjusted mean change in log-white matter hyperintensity volume for procalcitonin, 0.29; 95% CI, 0.13-0.44 and for MR-proANP, 0.18; 95% CI, 0.004-0.36). **CONCLUSIONS:** Higher concentrations of procalcitonin, a marker of infection, and MR-proANP, a marker of cardiac dysfunction, are independently associated with subclinical cerebrovascular damage. If further studies demonstrate an incremental value for risk stratification, biomarker-guided primary prevention studies may lead to new approaches to prevent cerebrovascular disease.

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**Procalcitonin and MR-proANP as biomarkers of subclinical cerebrovascular damage:
the Northern Manhattan Study**

Biomarkers of subclinical cerebrovascular damage

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Abstract

Background: Chronic infections and cardiac dysfunction are risk factors for stroke. We hypothesized that blood biomarkers of infection (procalcitonin, or PCT) and cardiac dysfunction (midregional pro-atrial natriuretic peptide, or MR-proANP), previously associated with small vessel stroke and cardioembolic stroke are also associated with subclinical cerebrovascular damage (SCVD), including silent brain infarcts (SBI) and white matter hyperintensity volume (WMHV).

Methods: The Northern Manhattan Study was designed to assess risk factors for incident vascular disease in a multiethnic cohort. A subsample underwent brain MRI and had blood samples available for biomarker measurement (n=1178). We used logistic regression models to estimate the odds ratios and 95% confidence intervals (OR, 95%CI) for the association of these biomarkers with SBI after adjusting for demographic, behavioral, and medical risk factors. We used linear regression to assess associations with logWMHV.

Results: Mean age was 70 ±9 years; 60% were women, 66% Hispanic, 17% black, 15% white. After adjusting for risk factors, subjects with PCT or MR-proANP in the top quartile, compared to the lowest quartile were more likely to have SBI (adjusted OR for PCT 2.2, 95%CI 1.3-3.7; for MR-proANP 3.3, 95%CI 1.7-6.3) and increased WMHV (adjusted mean change in logWMHV for PCT 0.29, 95%CI 0.13-0.44; for MR-proANP 0.18, 95%CI 0.004-0.36).

Conclusion: Higher concentrations of PCT, a marker of infection, and MR-proANP, a marker of cardiac dysfunction, are independently associated with SCVD. If further studies demonstrate an incremental value for risk-stratification, biomarker-guided primary prevention studies may lead to new approaches to prevent CVD.

Introduction

Subclinical cerebrovascular disease (SCVD), as manifested by subclinical brain infarcts (SBI) or white matter hyperintensities (WMH) visualized on MRI, has been associated with incident ischemic stroke¹ and cognitive dysfunction and dementia^{2, 3}.

There is very little data on blood biomarkers and their association with SCVD, but such knowledge could help identify novel preventive and therapeutic targets for stroke, cognitive dysfunction, and dementia simultaneously. For this purpose, the measurement of blood biomarkers, which may indicate underlying subclinical pathological processes, could be an important adjunct to traditional risk assessment.

Chronic infections have been associated with stroke risk and cognitive impairment even after adjusting for other potential confounders^{4, 5}. Serum procalcitonin (PCT) concentrations correlate with the extent and severity of microbial invasion⁶. In a recent study nested within in the Northern Manhattan Study (NOMAS), PCT concentrations were associated specifically with small vessel stroke⁷.

Higher natriuretic peptide levels reflect severity of cardiac dysfunction⁸. There is increasing evidence supporting the potential role of elevated natriuretic peptides as risk factors of cognitive impairment.⁹ Midregional pro-atrial natriuretic peptide (MR-proANP) has been shown to be associated specifically with cardioembolic stroke etiology^{7, 10}.

Both chronic infections and underlying cardiac dysfunction may be risk factors not only for overt ischemic stroke but also for SCVD. Thus we hypothesized that PCT, as a surrogate for bacterial infections, and MR-proANP, as a marker of cardiac dysfunction, previously associated with small vessel stroke and cardioembolic stroke⁷, respectively, would also be associated with MRI measures of SCVD in the ethnically diverse Northern Manhattan population.

Methods

Study population

NOMAS is a population-based cohort study among 3,298 initially stroke-free participants identified using random digit dialing with dual-frame sampling to identify telephone numbers. NOMAS was designed to evaluate the effects of medical, socioeconomic, and other risk factors on the incidence of vascular disease in a stroke-free multiethnic community cohort. Methods of participant recruitment, evaluation, and follow-up have been previously reported¹¹. Briefly, subjects were eligible if they: (1) had never had a stroke diagnosed; (2) were over age 40 years; and (3) resided in Northern Manhattan in a household with a telephone. Participants underwent a thorough baseline examination. Standardized questions about vascular risk factors were adapted from the Centers for Disease Control and Prevention Behavioral Risk Factor Surveillance System as defined previously.¹² Changes in health or vital status were determined through annual telephone follow-up and clinical examinations. The Institutional Review Boards at Columbia University Medical Center and the University of Miami approved the study. All participants gave informed consent to participate in the study.

MRI Sample

Participants included in this analysis were part of a sample recruited from the NOMAS cohort to undergo brain MRI scans, using the following criteria: (1) age ≥ 50 years; (2) no contraindications to MRI; and (3) willing to sign informed consent. To maximize recruitment, an additional 199 participants, who were household members of existing NOMAS participants (n=1091), were recruited into the MRI sample for a total of 1290 participants. Out of these 199 household members 29 (15%) were blood relatives. Imaging was performed on a 1.5T MRI system (Philips Medical Systems, Best, the Netherlands). Stored frozen serum samples, drawn at the time of the MRI, were available in 1178 subjects from this sub-cohort.

MRI examination

The processing of MRI scans in NOMAS has been published^{13, 14}. Briefly, the presence or absence of SBI was determined from the size, location, and imaging characteristics of the lesion¹⁵. Lesions were ≥ 3 mm in size with CSF density on the subtraction image, in a vascular distribution, and distinct from circle of Willis vessels and perivascular spaces if in the basal ganglia. Inter-observer agreement for SBI detection was 93.3%¹⁶.

Analyses for WMHV were performed using semi-automated measurements of pixel distributions and mathematical modeling of pixel-intensity histograms for cerebrospinal fluid and brain (white and gray matter) to identify the optimal pixel-intensity threshold to distinguish cerebrospinal fluid from brain matter. Analyses were performed using a custom-designed image analysis package (QUANTA 6.2 using a Sun Microsystems Ultra 5 workstation). WMHV was calculated as percent total intracranial volume (ICV) to correct for differences in head size¹⁷ and log-transformed to achieve a normal distribution (log-WMHV). All analyses were performed blind to participant identifying information and biomarker measurements.

Biomarker measurements

At baseline, blood samples were obtained, centrifuged, and frozen at -80°C in 1 ml aliquots until the time of analysis. The samples of 1178 subjects with available serum were shipped on dry ice to the Department of Clinical Chemistry, University Hospital of Zurich, for biomarker measurements. Samples were thawed at 4 °C prior to measurements and analyzed within one hour, thus reducing pre-analytic errors. PCT concentrations were measured using a sensitive assay with a detection limit of 0.007 ng/ml (B·R·A·H·M·S-us PCT sensitive KRYPTOR, Thermo-Scientific, BRAHMS, Germany). MR-proANP concentrations were measured using an immunoassay with a detection limit of 2.1 pmol/L (B·R·A·H·M·S

KRYPTOR, Thermo Scientific, BRAHMS, Germany). Quality control was maintained using standardized procedures. All testing was performed in a batched analysis, blinded to all clinical data. Stability at room temperature and after freezing and thawing cycles has been documented for both biomarkers ^{18, 19}.

Statistical Analyses

We calculated descriptive statistics for the clinical, demographic, and biomarker variables. The primary outcomes for this analysis were 1) the presence of SBI (dichotomous variable) and 2) WMHV (continuous variable). The main variables, PCT and MR-proANP, were log transformed to achieve linearity and analyzed continuously and by quartile in order to facilitate clinical interpretation. Odds ratios (OR, for SBI) and beta-coefficients (β , for WMHV) and 95% confidence intervals (95% CI) were calculated, unadjusted and adjusted for demographic and vascular risk factors. Covariates included predictors of ischemic stroke and WMHV in prior analyses in NOMAS, including age at the time of MRI, sex, race-ethnicity, education, and insurance status, physical activity, smoking status, moderate alcohol consumption, diabetes mellitus, cardiac disease including atrial fibrillation, systolic and diastolic blood pressure, low density lipoprotein (LDL), and high density lipoprotein (HDL). We also included eGFR, since these biomarkers undergo renal clearance. We assessed potential effect modification of the association between biomarker levels and the outcomes by each vascular risk factor.

All testing was two-tailed and $p < 0.05$ was considered statistically significant. All calculations were performed using SAS v9.1.3 (SAS Institute, Cary, NC).

Results

Baseline characteristics

Demographics and clinical characteristics of the study population (n=1178) are summarized in Table 1. The mean age of the sample at the time of brain MRI was 69.8 +/- 8.9 years, 60% were women, and the majority (66%) were Hispanic. Median PCT levels were 0.0254 ug/L (IQR 0.015-0.039 ug/L) and median MR-proANP levels were 90.2 pmol/L (IQR 62.2-132.8 pmol/L).

In this MRI cohort with available blood, 168 participants (15%) had at least one SBI and the median WMHV (WMHV/TCV*100) was 0.35% ICV (IQR 0.21-0.75 %ICV).

Biomarker distribution according to the presence of SBI is shown in figure A and B.

Association of PCT with MRI measures of SCVD

In the unadjusted analysis, PCT concentration was associated with prevalence of SBI (OR 1.32 per SD of logPCT, 95%CI 1.12-1.56). This association remained after adjusting for demographic and vascular risk factors (OR 1.25 per SD of logPCT, 95 %CI 1.03-1.50). When analyzed by quartiles, individuals in the top PCT quartile were more likely to have SBI compared to those in the lowest quartile (unadjusted OR 2.44, 95% CI 1.51-3.96) (Table 2). After adjusting for demographic and vascular risk factors, those with PCT in the top quartile, compared to the lowest quartile, remained more likely to have SBI (adjusted OR 2.16, 95% CI 1.26-3.69; Table 2). We found no effect modification by vascular risk factors.

Greater PCT concentration was also associated with greater WMHV (unadjusted mean change in logWMHV per SD of logPCT 0.18, 95%CI 0.13-0.24), and this association remained after adjusting for demographic as well as for vascular risk factors (adjusted mean change in logWMHV per SD of logPCT 0.10, 95%CI 0.04-0.15). Individuals in the top PCT quartile, compared to the lowest quartile, also had greater WMHV (unadjusted mean change in logWMHV 0.52, 95%CI 0.37-0.68). This association remained after adjusting for

demographic as well as vascular risk factors (adjusted mean change in logWMHV 0.29, 95%CI 0.13-0.44, Table 3). We found no effect modification by vascular risk factors.

Association of MR-proANP with measures of SCVD

In the unadjusted analysis MR-proANP concentration was associated with SBI (OR 1.67 per SD of log MR-proANP, 95%CI 1.42-1.97); this association remained after adjusting for demographic and vascular risk factors (OR 1.54 per SD of log MR-proANP, 95 %CI 1.22-1.94). When analyzed by quartiles, individuals in the top MR-proANP quartile were more likely to have SBI compared to those in the lowest quartile (unadjusted OR 3.99, 95% CI 2.39-6.65). This association remained after adjusting for demographic and vascular risk factors (adjusted OR 3.31, 95% CI 1.74-6.32; Table 2). We found no effect modification by vascular risk factors.

MR-proANP concentration was further associated with WMHV (unadjusted mean change in logWMHV per SD of logMR-proANP 0.28, 95%CI 0.22-0.33), though this linear association was no longer statistically significant after adjusting for demographic and vascular risk factors (adjusted mean change in logWMHV per SD of logMR-proANP 0.05, 95%CI -0.01-0.12). When analyzed by quartiles, participants with MR-proANP levels in the top quartile had greater WMHV than those in the lowest quartile (unadjusted mean change in logWMHV 0.74, 95%CI 0.59-0.90) and this persisted after adjusting for sociodemographic and vascular risk factors, though the effect was attenuated (adjusted mean change in logWMHV 0.18, 95%CI 0.004-0.36, Table 3). We found no effect modification by baseline vascular risk factors.

Discussion

In this urban, multiethnic, population-based sample, PCT, a marker of bacterial infection, and MR-proANP, a marker for **cardiac dysfunction**, were each independently

associated with two measures of subclinical cerebrovascular disease, SBI and WMHV.

However, the association of MR-proANP concentrations with WMHV was less pronounced compared to the association with SBI.

Silent brain infarction serves as an imaging biomarker of vascular brain health. SBI has been associated with traditional vascular risk factors, as well as with future clinical stroke²⁰, cognitive decline, and dementia^{3, 21}. The independent association of SBI with future stroke, however, after adjusting for vascular risk factors, suggests that SBIs reflect either an overall effect of uncontrolled vascular risk factors better than the presence or absence of each individual factor, or that other, yet unknown, factors play a role in the association with stroke and dementia. These unknown factors may include underlying chronic infection, measured by PCT, or subclinical cardiac disease, measured by MR-proANP.

Recent findings in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) suggest that mechanisms of SBI and white matter hyperintensities are intimately connected²². In these patients the majority of incident lacunes developed proximal to a white matter hyperintensity along the course of perforating vessels supplying the respective brain region²². With the caveat that not all SBI or WMH defined by MRI are definitely of vascular origin, this finding provides indirect evidence that both imaging markers are good surrogates of small vessel disease including consecutive overt strokes and eventually vascular dementia.

Procalcitonin was first shown in the early 1990s to be a sepsis-induced protein detectable in patient plasma²³. Currently PCT is used to guide antibiotic use in patients with lower respiratory tract infections.²⁴ If PCT concentrations are above 0.5 ug/L, acute bacterial pneumonia is very likely, and antibiotic treatment is strongly recommended. PCT synthesis and secretion are up-regulated by lipopolysaccharides and certain bacteria-specific pro-inflammatory mediators (for example, interleukin-1 β , tumor necrosis factor- α , and interleukin-6), and are down-regulated as concentrations of these substances decrease during

recovery from infection^{25,26}. Interestingly, a polymorphism in the gene encoding the monocyte receptor for bacterial lipopolysaccharide (CD14) was specifically associated with large atherosclerotic and small vessel stroke, but not other types of stroke²⁷. In a pilot case-cohort analysis in NOMAS we found an association of PCT with stroke risk, and in particular with small vessel stroke. The biological role of PCT *in vivo* at low concentrations in stroke-free people has so far been largely unexplored. Based on our data from this and our prior analysis⁷ we propose that even low concentrations of PCT may reflect ongoing subclinical inflammatory processes triggered by bacterial endotoxins, which in turn contribute to the small vessel damage that causes some SCVD,^{28 29} as well as overt small vessel stroke.

Brain natriuretic peptide (BNP) and N-terminal pro-B-natriuretic peptide (NTproBNP), as members of the family of the natriuretic peptides, have also been associated with incident stroke in some studies,^{30, 31} as well as with WMH and SBI in the Atherosclerosis Risk in Communities (ARIC) study. Additionally, higher NT-proBNP concentrations were prospectively associated with increased incident SBIs and WMH progression over 8 years of follow-up³². Our search did not reveal prior studies of MR-proANP, another member of the family of the natriuretic peptides, in relation to SBI or WMHV. Concerning mortality in patients with chronic heart failure, MR-proANP outperformed BNP and NT-proBNP. The proportion of explained variance showed that MR-proANP (4.36%) was a significantly stronger predictor of death than either NT-proBNP (2.47%, $p < 0.0001$) or BNP (2.42%, $p < 0.0001$). Both a new assay technology and the high biological stability of MR-proANP are potential explanations for these findings³³. The pathophysiological mechanism explaining the independent associations of MR-proANP with SBI and to a lesser extent with WMHV in our study needs confirmation. We hypothesize that high MR-proANP concentrations indicate the presence of underlying early atrial pathology, thus leading to silent strokes, but only to a slight increase in WMHV, which is less likely to have exclusively a cardioembolic etiology and could also reflect effects such as relative hypoperfusion primarily based on other

pathomechanisms. White matter lesions can occur with acute³⁴ and also chronic cerebral ischemia³⁵, but non-arteriolar and non-ischemic mechanisms for WMH have also been proposed³⁶, including venous sclerosis with subsequent venous hypertension³⁷, all of which are less prone to be associated with an underlying cardiac disease. The association of MR-proANP with WMHV, compared to the association with SBI, was thus more attenuated after adjustment, pointing to the fact that other comorbidities such as smoking are probably more important risk factors for an increase in WMHV.

Our study has limitations. Our cross-sectional analysis does not allow for determining causal relationships, and we cannot exclude the possibility of residual confounding. Our findings require replication in an external sample to ensure validity. Also, repeated measurements of the selected biomarkers and their change over time may be a better indicator of SCVD than levels measured at a single baseline point. Further, we did not perform direct comparison of PCT with other markers of infection or inflammation, such as C-reactive-protein, nor did we compare MRproANP with NT-proBNP. However in a previous study of high-sensitivity CRP in NOMAS, we did not find a significant independent association of CRP with incident stroke³⁸. Similarly, in a prior analysis of a subgroup of the NOMAS MRI cohort including different inflammatory markers, CRP was not independently associated with silent infarcts or white matter disease after adjusting for other risk factors and biomarkers³⁹. Finally, the blood samples were stored at -80 °C for several years, which could lead to some protein degradation. Degradation would, however have affected subjects with and without SBI similarly, and probably would have biased our results toward the null.

The strengths of this study include the population-based multiethnic cohort, including a large proportion of Hispanics who are frequently underrepresented in other cohort studies, and detailed clinical information on participants, permitting us to adjust for numerous potential covariates. Finally, this study provides prospectively planned imaging sequences to

specifically assess subclinical cerebrovascular disease as an important risk factor of overt stroke and dementia.

In conclusion, we report associations of PCT and MR-proANP with SCVD. The present study was aimed at identifying novel markers reflecting different potential mechanisms that may contribute to subclinical vascular disease. Whether these markers will be of clinical use in the future remains to be determined. If, however, other studies confirm these associations, and if further studies show an incremental value for risk stratification, we may in the future base the selection for specific primary preventive interventions concerning underlying subclinical infections and heart disease on these biomarker levels. Given the multiple adverse health outcomes associated with SCVD in older individuals these biomarker-guided interventions may have a clinically relevant impact and may be beneficial even for the prevention of both stroke and cognitive impairment.

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Figure legend

Figure A: Distribution of logPCT concentrations according to the presence of silent brain infarcts (SBI).

Figure B: Distribution of logMRproANP concentrations according to the presence of silent brain infarcts (SBI).

Table 1. Baseline demographics and risk factors of the Northern Manhattan Study Magnetic Resonance Imaging subsample with serum samples available for this analysis (n = 1178)

<u>Socio-demographic characteristics</u>		mean (+/- SD) or median (IQR); n (%)
Age at the time of MRI in years		69.8 (+/-8.9)
Women		707 (60%)
Race-Ethnicity		
	Hispanic	780 (66%)
	Non-Hispanic Black	200 (17%)
	Non-Hispanic White	171 (15%)
	Other	25 (2%)
Less than high school education		632 (54%)
Medicaid or no insurance		555 (47%)
<u>Medical Co-morbidities</u>		
Current tobacco use		184 (16%)
Moderate alcohol use*		484 (41%)
Mean systolic blood pressures in mmHg		137 (+/- 8)
Mean diastolic blood pressures in mmHg		78 (+/-10)
Estimated glomerular filtration rate		78 (+/-20)
Cardiac Disease †		
Diabetes mellitus ‡		228 (19 %)
<u>Laboratory parameters</u>		
High-density lipoprotein mg/dl		53.4 (+/-17)
Low-density lipoprotein mg/dl		115 (+/-35.3)
Procalcitonin ug/L		0.025(0.015-0.039)
MR-proANP pmol/L		90.2 (62.2-132.8)

*Moderate alcohol use = ≤ 2 servings of alcohol per day (reference all other groups)

† Cardiac disease= coronary artery disease, congestive heart failure and atrial fibrillation

‡Diabetes mellitus was defined as fasting blood glucose ≥ 126 mg/dl, the subject's self-report of diabetes mellitus, or insulin and/or hypoglycemic agent use.

Table 2. Association of biomarkers with silent brain infarcts

Parameter	Unadjusted	Model 1*	Model 2**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
PCT			
First quartile	Reference		
Second quartile	1.5 (0.9-2.6)	1.4 (0.8-2.4)	1.4 (0.8 - 2.4)
Third quartile	1.4 (0.9-2.4)	1.3 (0.7-2.2)	1.2 (0.7 - 2.2)
Fourth quartile	2.4 (1.5-4.0)	1.9 (1.1-3.2)	2.2 (1.3 - 3.7)
MR-proANP			
First quartile	Reference		
Second quartile	1.3 (0.8-2.4)	1.4 (0.8-2.6)	1.5 (0.8 - 2.8)
Third quartile	2.3 (1.4-4.0)	2.0 (1.1-3.6)	1.9 (1.1 - 3.6)
Fourth quartile	4.0 (2.4-6.7)	2.8 (1.5-5.2)	3.3 (1.7 - 6.3)

*Model 1; adjusted for age, gender, race-ethnicity, education and insurance status.

** Model 2; adjusted for covariates in Model 1 plus physical activity, smoking status, moderate alcohol consumption, cardiac disease, diabetes mellitus, systolic and diastolic blood pressure, low density lipoprotein (LDL), high density lipoprotein (HDL), estimated glomerular-filtration-rate (eGFR).

Table 3. Association of top quartile of PCT and MR-proANP with log-total white matter hyperintensity volume

Parameter	Univariate analysis parameter estimate (95% CI)	Model 1* parameter estimate (95% CI)	Model 2** parameter estimate (95% CI)
PCT			
First quartile	Reference		
Second quartile	0.20 (0.04-0.35)	0.08 (-0.06-0.22)	0.09 (-0.06-0.23)
Third quartile	0.31 (0.15-0.46)	0.21 (0.06-0.35)	0.19 (0.05-0.34)
Fourth quartile	0.52 (0.37-0.68)	0.29 (0.15-0.44)	0.29 (0.13-0.44)
MR-proANP			
First quartile	Reference		
Second quartile	0.08 (-0.07-0.23)	-0.02 (-0.16-0.13)	-0.03 (-0.17-0.12)
Third quartile	0.29 (0.14-0.44)	0.00 (-0.15-0.15)	-0.01 (-0.16-0.15)
Fourth quartile	0.74 (0.59-0.90)	0.22 (0.06-0.39)	0.18 (0.004-0.36)

*Model 1; adjusted for age, gender, race/ethnicity, education and insurance status.

**Model 2; adjusted for age, gender, race/ethnicity, education, insurance status, physical activity, smoking status, moderate alcohol consumption, cardiac disease, diabetes mellitus, systolic and diastolic blood pressure, low density lipoprotein (LDL), high density lipoprotein (HDL), estimated glomerular-filtration-rate (eGFR).